RESEARCH

New Mutations in a *Delta-9-Stearoyl-Acyl Carrier Protein Desaturase* Gene Associated with Enhanced Stearic Acid Levels in Soybean Seed

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] oil from conventional cultivars typically contains ~30 g kg^{-1} stearic acid of the total seed oil. Increased stearic acid concentration in the seed oil of soybeans is desirable for certain food and industrial uses. To date a small number of mutants have been developed with increased stearic acid levels three to six times that of normal. At least two such lines were found to possess separate mutations in the *delta-9-stearoyl-acyl carrier protein desaturase C* gene (*SACPD-C*) that dramatically increased seed stearic acid concentration. We now report additional independent mutations in this gene that increase seed stearic acid levels of the soybean germplasm line RG7 to ~116 g kg^{-1}. An F_5 recombinant inbred line (RIL) population was developed to determine the relationship between the RG7 *SACPD-C* mutation and stearic acid concentration. Transgressive segregation in the progeny of the cross between mutant lines RG7 and RG2 (low palmitic acid) further increases seed stearic acid to almost 180 g kg^{-1}, suggesting the presence of yet another gene having a significant effect on stearic acid accumulation. We also discerned an independent, presumably allelic, mutation within the *SACPD-C* gene in line RG8, which has about 106 g kg^{-1} stearic acid. Molecular markers diagnostic for the RG7 and RG8 *SACPD-C* mutations were developed, enabling rapid selection for these mutations in the development of cultivars with increased seed stearic acid content in the seed oil.

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Abbreviations: EMS, ethyl methane sulfonate; LG, linkage group; PCR, polymerase chain reaction; RIL, recombinant inbred line; SACPD, delta-9-stearoyl-acyl carrier protein desaturase; *SACPD*, *delta-9-stearoyl-acyl carrier desaturase* gene; SNP, single nucleotide polymorphism.

Soybean [*Glycine max* (L.) Merr.] has long been an important source of food and oil, with historical records of its use dating back some 4000 yr (Wang and Wang, 1992). Today soybeans are considered to be the world's most important oilseed crop, accounting for 58% of total oilseed production worldwide in 2010 (American Soybean Association, 2011). Soy oil is used not only for food purposes but also for a range of products in industry (Schmitz et al., 2008).

There is an increasing awareness of the health advantage of stearic acid over other saturated fatty acids in baking and other food industry products (Kris-Etherton and Yu, 1997; Thijssen et al., 2005). Stearic acid, unlike other saturated fats (including palmitic acid), has an apparently neutral effect on blood cholesterol concentration that is not very different from the effects of some of the mono-unsaturated fatty acids (Kris-Etherton and Yu, 1997). Moreover, when other saturated fats in controlled diets are replaced with stearic acid, there are demonstrated beneficial effects on low-density lipoprotein cholesterol levels in human blood serum (Hunter et al., 2010).

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In the automotive industry stearic acid has a major use in the production of rubber for tires and other rubber parts (Dogadkin and Beniska, 1958; Beniska and Dogadkin, 1959; Luyt, 1993). Stearic acid is also one of the most commonly used lubricants during injection molding and pressing of ceramic powders (Tsenga et al., 1999). Other uses of stearic acid include use as a lubricant additive in paper making (Flynn et al., 1996), as a component of defoaming compounds in certain manufacturing processes (Riggs and Wheeler, 1996), and as an epilame treatment applied to precision mechanical components of devices such as watches (Vineall, 1967). Therefore, the renewed confidence in the role of stearic acid in food products and the desire to produce more environmentally friendly vehicles (Ontario Biocar Initiative; http://bioproductsatguelph.ca/index.html) has led to a renewed interest in soybeans with high levels of stearic acid.

The enhanced levels of stearic acid in particular mutant soybean lines have been associated with molecular changes that negatively impact the activity of the delta-9-stearoyl-acyl carrier protein desaturase (SACPD) enzyme (Pantalone et al., 2002; Wilson, 2004), which inserts the first double bond into the 18-C fatty acid at C9. The gene encoding this enzyme has been localized to the *Fas* locus on linkage group (LG) B2 (chromosome 14) (Spencer et al., 2003). At least six allelic mutations have been documented that increase levels of stearic acid by varying amounts (Hammond and Fehr, 1983; Graef et al., 1985a, b; Rahman et al., 1997; Pantalone et al., 2002). The molecular basis of these mutations was unknown until recently.

Byfield et al. (2006) reported that two forms of SACPD (A and B) are produced by soybean plants and that these are variably regulated within the plant. The authors, however, did not show conclusively that the activity of the two forms accounted for the markedly different levels of seed stearic acid produced by mutant breeding lines. In 2008, Zhang et al. reported a third variant of SACPD (SACPD-C) that was highly expressed in the seed but showed little or no expression in other plant tissues. They found that in two mutant lines that accumulate enhanced levels of seed stearic acid, line FAM94-41 carried a critical *SACPD-C* mutation whereas *SACPD-C* was completely absent in line A6. The stearic acid concentration in the seed produced by progeny of a cross between FAM94-41 and N98-4445A (a mid-oleic line) could be directly correlated with inheritance of the *SACPD-C* mutation.

The ethyl methane sulfonate (EMS)-generated mutant lines RG7 and RG8 from the University of Guelph resemble FAM94-41 in that they exhibit moderately enhanced levels of stearic acid in the seed oil. Hou (2004) examined the characteristics of RG7 and concluded that a major recessive gene located on LG B2 controlled the accumulation of stearic acid, suggesting that the most probable gene involved was located in the *Fas* locus. In this paper we report two new mutations in the sequence of *SACPD-C* that significantly enhance seed stearic acid accumulation.

MATERIALS AND METHODS

Plant Materials

Soybean lines RG2, RG7, and RG8 were used in this study. RG2 has approximately normal levels of stearic (C18:0) and oleic acid (C18:1) but low palmitic acid (C16:0; 43 g kg^{-1}). It is derived from the cross CLP-1 × ELLP-2. CLP-1 was developed from a mutated population of C1640 (Wilcox et al., 1984). ELLP-2 was developed from 'Elgin 87' by EMS mutagenesis (Primomo et al., 2002). RG7 is another Elgin 87 mutant that has normal levels of palmitic acid but significantly enhanced levels of stearic acid (120 g kg^{-1}) and slightly reduced levels of oleic acid (156 g kg^{-1}). RG8 is a line with elevated stearic acid (106 g kg^{-1}) and reduced linolenic acid (C18:3; 48 g kg^{-1}). RG8 was created by an EMS mutagenesis treatment of the reduced linolenic mutant (*fan* locus) line C1640 (44 g kg^{-1} linolenic acid) (Primomo et al., 2002), and C1640 was generated by EMS mutagenesis of 'Century' (Wilcox et al., 1984). RG2 and RG7 were crossed and 115 F_5 recombinant inbred line (RIL) sibs were developed by single seed descent from the F_2 stage. The parents and 114 of the RILs were grown at Ridgetown, ON, in 2001, in three-row plots 2.69 m long and spaced 47 cm apart, with two replications. Further details are available in the thesis by Hou (2004).

Oil Profile Analysis

Samples of 20 to 30 seeds of both parents and the RILs were randomly selected from the seed harvested at Ridgetown, ON in 2001. Seeds were dried overnight in an oven at 110°C and ground to fine flour for 3 min using a coffee grinder (Moulinex). Oil from approximately 0.09 g of this flour was extracted as fatty acid methyl esters using the Gas Chromatography Vegetable Oil Fatty Acid Composition method (AOCS, 2009). Fatty acid methyl esters were dissolved in 4 mL iso-octane. Samples were analyzed by a Hewlett-Packard 6890 series gas chromatograph (Agilent Technologies, Inc.) fitted with a DB-23 15 m by 0.25 mm (i.d.) column (J&W Scientific). Operating conditions were 2 μL injection volume and He carrier gas at 0.7 mL min^{-1}, temperatures of 290 and 330°C for the injector port and decay-spin echo procedure detector, respectively, and oven temperatures ramped from 180 to 240°C over a 9-min interval. Chromatograms were analyzed using HP ChemStation software (Agilent Technologies, 2010), with individual fatty acids presented as a percentage of total oil content.

Gene Sequencing and Analysis

Total genomic DNA was extracted from young leaf material of the two parent lines (RG2 and RG7) and 114 F_5 RILs grown in a growth room at the University of Guelph using a GenElute Plant Genomic Miniprep Kit (Sigma-Aldrich Corp.). Four to five plants of each RIL were sampled and tested individually. Comparisons were made of *SACPD* gene sequences A, B, C in the parents (RG2 and RG7) by means of polymerase chain reaction (PCR) amplification and sequencing. Primer sequences used for *SACPD-A* and *SACPD-B* were from Byfield et al. (2006) and for *SACPD-C* from Zhang et al. (2008). The reactions were performed using the conditions described by Byfield et al. (2006) and Zhang et

al. (2008). Polymerase chain reaction conditions for types A and B were optimized for use on a Stratagene Robocycler 96 (Agilent Technologies), with the resultant thermocycling parameters for a 30 μL reaction: 94°C incubation for 2 min followed by 35 cycles of 94°C for 90 s, 55°C for 3 min, and 72°C for 3 min followed by a final 72°C extension of 7 min. For type C, conditions used were 95°C incubation for 2 min followed by 30 cycles of 94°C for 1 min, 55°C for 90 s, and 72°C for 2 min followed by a final 72°C extension of 7 min. Polymerase chain reaction products were visualized on a 1.5% agarose gel run at 120 V for 1.5 h and stained with ethidium bromide. After band excision, PCR products were cleaned using a Sigma GenElute Gel Extraction Kit (Sigma-Aldrich Corp.). Polymerase chain reaction products were cloned into the pCR4-TOPO vector using a TOPO TA Cloning Kit (Invitrogen Canada Inc.) and recovered after multiplication (under manufacturer's recommended conditions) using a GenElute Plasmid Miniprep Kit (Sigma-Aldrich Corp.) Sequencing reaction volumes were 15 μL using 1 μL BigDye Terminator v 3.1 ready reaction pre-mix, 1 μL BigDye sequencing buffer (Applied Biosystems, Inc.), 10 pmol primer, and 20 to 50 ng template DNA. Cycle sequencing reactions conditions were 96°C for 2 min followed by 30 cycles of 96°C for 30 s, 50°C for 15 s, and 60°C for 4 min. Sequencing of plasmid inserts was completed at the University of Guelph Genomics Facility using an Applied Biosystems 3730 DNA analyzer. Sequences were compared with previously published gene sequences of this species.

Total genomic DNA was extracted from RG8 seed with a DNeasy Plant Mini Kit (Qiagen, Inc.) using ~20 to 30 mg seed tissue and DNA was used at ~5 to 50 ng per PCR amplification or Simpleprobe assay. Primer sequences for *SACPD-C* were taken from Zhang et al. (2008). Polymerase chain reaction amplification was performed using Ex taq DNA polymerase (Takara) according to manufacturer's recommendations in a PTC-200 thermocycler (MJ Research/Bio-Rad) with the following conditions: 95°C for an initial 5 min denaturation followed by 40 cycles of 95°C for 30 s and 60°C for 30 s and an extension step at 72°C for 1 min kb^{-1} of target sequence. Polymerase chain reaction products were run on 1% agarose gels to ensure appropriate size and purified using a QIAquick PCR Purification Kit (Qiagen, Inc.). Following purification, products were Sanger sequenced at the DNA Core Facility at the University of Missouri-Columbia.

Point Mutation Analysis

For the analysis of a point mutation in *SACPD-C* (RG7), new primers were designed using NetPrimer (Premier Biosoft International) to encompass the polymorphism, producing a fragment 300 bp long. The primer sequences were as follows: RG7-single nucleotide polymorphism (SNP)-F: 5′-CCTTCCACAACTTCCGTGTTCTTCTA-3′; RG7-SNP-R: 5′-TCATCAGGTAACTCTTTAGTGCGTTCG-3′. Polymerase chain reaction conditions were 15 μL total volume and incubation for 2 min at 94°C followed by 30 cycles at 94°C for 60 s, 58°C for 90 s, and 72°C for 2 min with a final extension of 5 min at 72°C. Other PCR components were as described above for sequencing reactions.

The restriction enzyme *Hae*III, used to digest the amplified product at the point mutation, was chosen using RestrictionMapper (http://www.restrictionmapper.org/). *Hae*III recognizes the sequence GGCC, cutting the DNA sequence between G and C. The 114 RILs were subjected to PCR amplification of the region surrounding the point mutation using primers RG7-SNP-F and RG7-SNP-R followed by digestion of the product by *Hae*III in accordance with the manufacturer's instructions (Takara Biotechnology Co. Ltd.). Digest products were separated on a 1.5% agarose gel run at 120 V for 1.5 h, stained with ethidium bromide, and visualized under ultraviolet light.

SimpleProbe Assays

SimpleProbe oligonucleotides (Roche Applied Sciences) were designed using the Lightcycler Probe Design Software, version 1 (Roche Applied Science, 2004) to be exactly complimentary to the 'Williams 82' reference sequence (Schmutz, et al., 2010). SimpleProbes were purchased from Roche Applied Sciences.

All SimpleProbe assay asymmetric PCR reactions contained DNA template, buffer (40 mM Tricine KOH [pH 8.0], 16 mM KCl, 3.5 mM $MgCl_2$, 3.75 μg mL^{-1} bulk segregant analysis, and 200 μM deoxyribonucleotide triphosphates), 10% (v/v) dimethyl sulfoxide, 0.5 μM of the primer corresponding to the amplified DNA strand complimentary to the probe, 0.1 μM of the primer from the same sense strand as the SimpleProbe, 0.2 μM SimpleProbe, and 0.2X Titanium Taq polymerase (BD Biosciences). SimpleProbe genotyping reactions were performed using a Lightcycler 480 II (Roche Applied Sciences) with the following conditions: 95°C for an initial 5 min denaturation followed by 45 cycles of 95°C for 30 s and 60°C for 30 s and an extension step at 72°C for 30 s. A negative control was included to verify that no genomic contamination of stocks was present. Following asymmetric PCR, melting curve analysis was performed on the LC480 II, using the following conditions: reactions were heated to 95°C for 5 min followed by a 2 min hold at the lowest temperature to be evaluated by melting curve. Melting curve analysis was performed with 10 readings collected per 1°C and covered the following ranges: 50 to 75°C for *SACPD-C*-RG7 and 55 to 75° for *SACPD-C*-RG8 (Table 1).

RESULTS

Oil Profiles

A population of RILs developed from RG2 and RG7 was evaluated along with their parents for their fatty acid profiles. The RG2 and RG7 parental lines differed markedly from each other in fatty acid composition for the all the major fatty acids, with the exception of linolenic acid. The oil profiles and fatty acid ranges of the field-grown parental lines and RILs are provided in Table 2.

The percentage of palmitic acid appeared to be largely independent of stearic acid ($r = -0.20$). Stearic acid and oleic acid showed a strong negative correlation ($r = -0.87$). Weaker correlations were observed for stearic and linoleic acid ($r = -0.62$) and stearic and linolenic acid combined ($r = 0.42$). There was a positive correlation between oleic and linolenic acid concentrations ($r = 0.65$) and a weaker correlation between concentrations of oleic and linoleic acid ($r = 0.35$) combined. There was no apparent correlation between the seed concentration of linoleic and linolenic acid ($r = 0.02$). RG2 was significantly lower in

Table 1. SimpleProbe reactions contained the following primers, with SimpleProbe Chemistry (SPC) corresponding to the proprietary SimpleProbe quencher sequence.

SimpleProbe-related primers		
Primer name†	Primer sequence (5′-3′)	Primer concentration
SACPD-C/RG7_F	ATCTCCAACCTCTCCACAGTTC	0.5 μM
SACPD-C/RG7_R	TCAGGGAGGAAGTTTTGTGG	0.1 μM
RG7 Simpleprobe	Fluorescein-SPC-GGCCCATCCCTCCAAGGAC-Phosphate	0.2 μM
SACPD-C/RG8_R	CCACCGTTCAACGAGAAACT	0.5 μM
SACPD-C/RG8_F	GCCATAGGGAACATGATGGA	0.1 μM
RG8 Simpleprobe	Fluorescein-SPC-TGCCGGCGCACCTTATGTAC-Phosphate	0.2 μM

†SACPD, delta-9-stearoyl-acyl carrier protein desaturase gene

Table 2. Fatty acid profiles of RG2, RG7, and 115 recombinant inbred lines (RILs) when grown in the field at Ridgetown, ON, in 2001 (Hou, 2004).

	Seed oil (g kg^{-1})		
Fatty acid	RG2	RG7	RILs
Palmitic	43.9	97.7	33.5–113.8
Stearic	31.4	116.1	31.4–178.7
Oleic	234.2	178.8	149.8–292.4
Linoleic	597.1	508.3	487.6–597.3
Linolenic	93.4	99.1	76.4–112.9

palmitic acid than RG7, which has normal levels, and RILs showed transgressive segregation for both stearic acid and oleic acid far exceeding the high parent values (Table 2).

Gene Sequencing and Analysis

Gene sequences of *SACPD* types A, B, and C were amplified by PCR as described above, with the exception that for Type A only segments differing from Type B were amplified and sequenced. No differences were found within the sequences of exons of Type B or the amplified regions of Type A to those previously published (Byfield et al., 2006).

For *SACPD-C* of RG7, a point mutation was found at position 192 (with respect to the *SACPD-C* start codon) where a G→A substitution resulted in changing a tryptophan residue to a stop codon at position 64 of the predicted amino acid sequence (Fig. 1), causing premature termination of the protein product after only 63 amino acids. No mutations were found within this gene for RG2. For *SACPD-C* of RG8, a point mutation was found within exon two at position 857 (C→T), which results in the substitution of a leucine residue for an ancestrally invariant proline residue (P286L) (Fig. 3).

Point Mutation Analysis of RILs

The amplification of a 300 bp segment of *SACPD-C* in the region surrounding the RG7 mutation and the subsequent digestion of this PCR product permitted the classification of RILs as either homozygous for the absence or presence of the mutation or heterozygous. Nonmutant lines had two restriction sites at positions 101 and 155, producing three fragments 54, 101, and 145 bp long whereas mutant lines had just one restriction site at position 101, resulting in two fragments of 101 and 199 bp. Heterozygous lines produced all four of the above fragments.

Forty-eight RILs were found to be homozygous for the wild-type (nonmutant) allele, and another 57 were

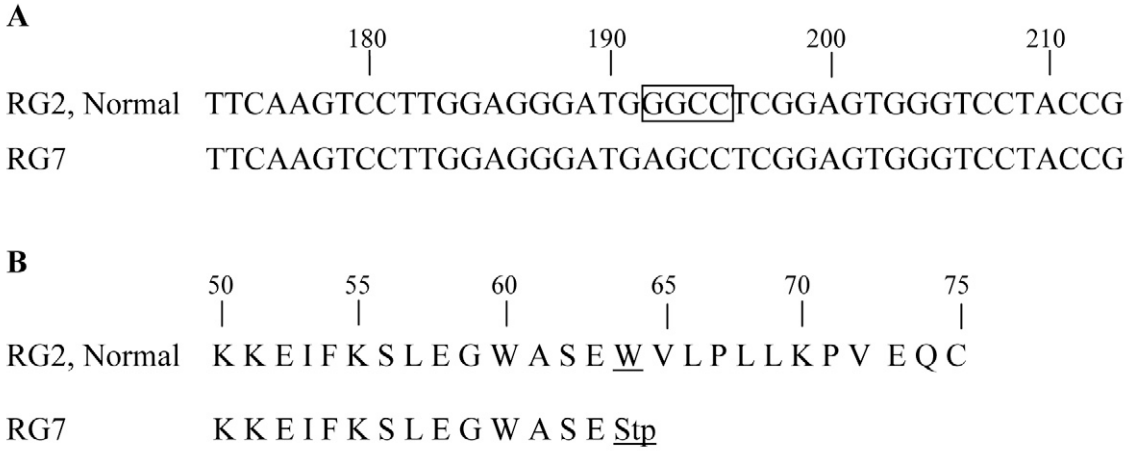

Figure 1. Point mutation in the *delta-9-stearoyl-acyl carrier protein desaturase C* gene (*SACPD-C*) of RG7. (A) Sequence comparison of the *SACPD-C* genes from soybean lines RG2 (normal stearic) and RG7 in the region surrounding the G→A polymorphism at position 192 (with respect to the initiator codon of *SACPD-C*). A rectangle denotes the recognition sequence of restriction enzyme *Hae*III that is absent in the sequence of RG7. (B) Localized amino acid sequence comparison showing the tryptophan to stop codon (underlined) substitution in the predicted SACPD-C enzyme from RG7. Amino acid positions are in reference to the initiator start methionine residue.

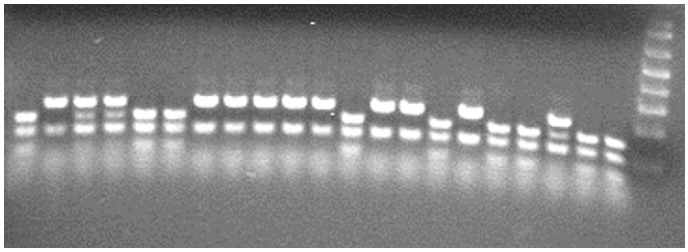

Figure 2. Agarose gel of the amplified 300 bp region of the RG7 single nucleotide polymorphism for F_5 recombinant inbred lines of the soybean cross RG2 × RG7 after digestion with *Hae*III. The three alleles are homozygous normal (lanes 1, 5, 6, 12, 15, 17, 18, 20, and 21), homozygous mutant (lanes 2, 7–11, 13, 14, and 16), and heterozygous (lanes 3, 4, and 19). Fragment sizes produced are 54, 101, 145, and 199 bp. On the right is a 1 kb+ ladder with bands at 100 bp intervals to 500, 650, 850, and 1000 bp.

homozygous for the mutant allele. Nine RILs were found to be heterozygous. The three genotypes could be clearly distinguished on a 1.5% agarose gel, as shown in Fig. 2.

Analysis of RIL sibs showed a very strong correlation between presence of the RG7 mutation and above normal (~54 g kg^{-1}) stearic acid concentration (r = 0.98). All RILs exhibiting in excess of 64 g kg^{-1} stearic acid were found to carry the mutation whereas all those with less than 58 g kg^{-1} stearic acid did not. One RIL exhibiting a stearic acid concentration of 64 g kg^{-1} did not carry the mutation. Lines found to be heterozygous for the mutation had stearic acid concentrations ranging from 58 to 111 g kg^{-1} whereas those homozygous for the mutation all had stearic concentrations in excess of 72 g kg^{-1}. Forty-five of the 57 RILs carrying the (homozygous) mutant allele exhibited stearic acid levels above that of the mutant parent RG7 (data not presented).

Development of Molecular Marker Assays to Directly Select for RG7 and RG8 *SACPD-C* Mutations

Development of a suitable marker for breeders to detect the mutation of *SACPD-C* carried by RG7 or RG8 facilitates selection for the gene in a segregating population. We developed a simple cleaved amplified polymorphic sequence marker that allows us to effectively distinguish between plants carrying the RG7 mutation in either the heterozygous or homozygous conditions and those not carrying the mutation. With access to only a thermocycler, appropriate primers or reagents, and agarose gel electrophoresis equipment, effective genotyping of individual DNA samples can be performed on a moderate throughput basis.

To generate high throughput genotyping methods, we also developed two SimpleProbe (Roche Applied Sciences)-based assays for the RG7 and RG8 *SACPD-C* mutations. SimpleProbe genotyping assays rely on the disassociation kinetics of a self-quenching, fluorescently labeled oligonucleotide. Although this technique requires a real-time PCR instrument, it consists of a single closed-tube PCR reaction and melting curve analysis. As such, genotyping results for several hundred DNA samples are available within 2 h, with no need to open PCR tubes or run agarose gels. Examples of typical SimpleProbe genotyping reactions are shown in Fig. 3.

DISCUSSION

Hou (2004) previously suggested that a reduction in activity of a gene located at the *Fas* locus (SACPD) was the most probable reason for elevated stearic acid in the seed of RG7, although this could not be confirmed. Byfield et al. (2006) found that the expression of *SACPD-A* and *SACPD-B* was measurable in the developing seed of three lines—'Dare' (normal), A6 (high stearic), and N01-3544 (mid-oleic)—and that *SACPD-A* and *SACPD-B* expression decreased under warmer conditions (Byfield and Upchurch, 2007). Therefore, while no correlations have

Figure 3. Typical SimpleProbe-based melting curve genotyping for the *delta-9-stearoyl-acyl carrier protein desaturase C* gene (*SACPD-C*) mutations found in RG7 (A) and RG8 lines (B). Homozygous wild-type (WT) samples are represented by the higher melting temperature peak to the right (WT for both is ~65.5°C) and homozygous mutant lines feature only a single peak to the left (mutant RG7 ~57°C; mutant RG8 ~56°C). Heterozygotes feature both WT and mutant peaks. NCBI, National Centre for Biotechnology Information.

been made between either *SACPD-A* or *SACPD-B* and seed stearic acid, the discovery and characterization of a third soybean *delta 9-stearoyl-acyl carrier protein desaturase* gene, *SACPD-C*, led to the finding that the high stearic acid mutant A6 lacks a functional copy of the *SACPD-C* gene, which defines the *Fas* locus (Zhang et al., 2008).

We have found a nonsense mutation within the coding region of *SACPD-C* in line RG7 that has resulted in the insertion of a stop codon at position 64 of the predicted amino acid sequence. The presence or absence of the mutation in the homozygous form shows a close correlation with increased stearic acid in the oil. The trend for heterozygous lines is less clear and is probably a consequence of seed admixtures caused by machine harvesting the trial. Likewise, the one RIL with 64 g kg^{-1} stearic acid may in fact have carried the mutant gene but could have tested negative for the mutation as a result of an accidental admixture. We also investigated another line with elevated stearic acid, RG8, and determined that it bears a novel, independent mutation affecting an absolutely ancestrally invariant proline residue within the SACPD-C protein (Fig. 3). Seed oil from RG7 and RG8 plants accumulate similar levels of stearic acid, despite one containing a null allele of *SACPD-C* and the other a missense mutation affecting the same gene.

There is no evidence that the 63 amino acid product of the null allele of RG7 *SACPD-C* has any potential to have enzyme activity. It appears, however, that some SACPD activity remains despite the null allele, as the desaturation of stearic acid is moderately reduced, with seed stearic acid levels in RG7 raised from "normal" levels of 20 to 54 g kg^{-1} (Garde and White, 2008) to 116 g kg^{-1}, unlike those of A6. A6 has no functional *SACPD-C* and develops seed stearic acid concentrations of 300 g kg^{-1} (Zhang et al., 2008). This could perhaps also be expected of RG7 if, in fact, *SACPD-C* is the main determinant of stearic acid in the seeds and is nonactive, as the sequencing results suggest.

In a previous study, Primomo et al. (2002) reported a mean of 120 ± 2.4 g kg^{-1} seed stearic acid across 12 environments in Ontario in 3 yr (1996–1998) for RG7. Likewise, for RG8 they reported a mean for stearic acid concentration of 106 ± 2.4 g kg^{-1} for the same sites and time period (Primomo et al., 2002). These results compare favorably to that reported for FAM94-41 (90 g kg^{-1}), which has a mutation in the same gene resulting in the substitution of aspartate for asparagine at position 126 of the predicted amino acid sequence (Zhang et al., 2008).

The mutations for high stearic acid in A6 and FAM94-41 are allelic (Pantalone et al., 2002) and in the same gene as that of the RG7 and RG8 mutations (Zhang et al., 2008), making it probable that the four are allelic. Therefore a cross between any of these lines is unlikely to increase the range of stearic levels already achieved. An examination of the fatty acid profiles of the RILs from the cross between RG2 and RG7 showed significant transgressive segregation for stearic acid, with the highest observed level of stearic acid being in excess of 175 g kg^{-1} (Table 2). It appears that there may be one or more genes acting in concert with the RG7 *SACPD-C* mutation to increase stearic acid and that the allele of the gene(s) conferring the further increase in stearic acid was donated by RG2. Interestingly, Zhang et al. (2008) reported a very similar outcome for the cross between the lines FAM94-41 (high stearic acid) and N98-4445A (mid-oleic acid) (Burton et al., 2006), with the highest stearic acid levels of F_2 progeny in the range of 130 to 150 g kg^{-1}. Both RG2 and N98-4445A have some reduction in the levels of palmitic acid (43 and 90 g kg^{-1}, respectively), and it may be speculated that the gene causing the reduction in palmitic acid is also involved in increasing stearic acid in mutant progeny lines.

The high (normal) level of palmitic acid in RG7 is not considered good for human health. Therefore, the combination of lines RG7 or RG8 with RG2 in a high-stearic, low-palmitic acid cultivar is desirable from both a food and industrial perspective—especially if the oxidatively unstable linoleic and linolenic fatty acids could be reduced or eliminated, either by simple processing or further breeding.

The mutations carried by RG7 and RG8 have the potential to be useful in breeding new high-stearic acid lines, especially when combined with the as yet unknown gene from RG2. RG7 has favorable yield and quality profiles, with seed yields approximately 94% of that of its Elgin87 parent and maturation 123 d after planting. RG8 has a slightly less favorable profile, with a yield approximately 72% of its parent Century and maturation 132 d after planting (Primomo et al., 2002). RG2 yielded approximately 65 to 68% as much as checks with a comparable maturity and matured 114 d after planting, so it is estimated to be a late maturity group II genotype (Hemingway, 2010).

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